ENZYMES IN INSECTS: ALKALINE PHOSPHATASE.

By Morris Rockstein and M. David Inashima,[1]
Pullman, Washington.

Introduction

In an earlier report, Rockstein and Levine (1951) presented data on the comparative activity of the acid phosphatase hydrolyzing sodium β-glycerophosphate for five species of insects. Chiefly histochemical, qualitative studies on the alkaline enzyme by Yao (1950a, b) on the developing embryo and preimaginal stages of *Drosophila melanogaster,* by Day (1949) on immature and adult stages of a variety of species of insects, by Bradfield (1946, 1951) on the goat moth larva (*Cossus cossus*), the silkworm (*Bombyx mori*), and several species of spiders, and by Denucé (1952) on two varieties of *Bombyx mori,* all tend to confirm Moog's (1946) summary of the possible role of alkaline phosphatase; in histodifferentiation, cuticle synthesis, transport across a gradient barrier, nucleic acid and silk synthesis, etc., in invertebrates as well as in vertebrates.

The object of this paper is to present a summary of comparative activity of the alkaline enzyme, studied at a pH of 8.1, in the same species in which the acid enzyme had been studied previously (Rockstein and Levine, *op. cit.*), as well as the large milkweed bug (*Oncopeltus fasciatus*), a species commonly employed in laboratory studies of a physiological or insecticidal nature. Data are also presented for each sex of the American roach (*Periplaneta americana*) as well as for the normal and resistant (Orlando #1 colony, topically exposed to DDT for 110 generations) strains of the house fly (*Musca domestica*).

Method

Instead of being decapitated, insects were collected and inactivated in a jar previously stored at –25° C, counted and weighed, and homogenized according to the method described by Rockstein and Herron (1951). Check determinations of acid enzyme activity were made at the same time, at a pH of 5.4. Also the number of individuals of *P. sericata* homogenized and made up to a final

[1] Laboratories of Zoophysiology, State College of Washington. This investigation was supported in part by funds provided for biological and medical research by the State of Washington Initiative Measure No. 171.

TABLE I

PHOSPHATASE ACTIVITY IN SEVERAL SPECIES OF INSECTS

Species	Age	Number of Individuals in 50 Ml.	Average Body Weight in Mg.	Measured Activity in μg. P/0.2 ml./1.5 Hr.		Activity in Mg. P/Gm./Hr.	
				Acid	Alkaline	Acid	Alkaline
Apis mellifera L.	< 22 hr. adult worker	20	109.6	8.08	7.77	1.84	1.78
	< 22 hr. adult drone	5	259.1	49.50	3.03	19.10	1.17
Musca domestica L.	< 20 hr. adult (N)	50	12.3	7.01	0.87	5.70	0.71
	< 22 hr. adult (R)	50	14.2	7.37	1.59	5.16	1.11
Tenebrio molitor L.	3.4 cm. larva	20	242.9	4.17	2.95	0.43	0.30
Oncopeltus fasciatus (Dall)	7–8 mm. nymph	17	21.5	2.32	0.95	3.18	1.31
	"adult"	20	40.4	7.22	1.58	4.46	0.98
Periplaneta americana (L.)	"adult" male	5	850.8	6.48	3.69	0.76	0.44
	"adult" female	5	944.8	6.20	2.67	0.66	0.28
Phaenicia sericata (Meig.)	< 22 hr. adult	50	13.9	0.71	0.98	0.51	0.71

diluted volume of 50 ml. was increased to 50 (over the 25 employed formerly) to obtain a low, but measurable, activity.

Results

Table I summarizes the pertinent data for the several species studied. (N) and (R) house flies refer to normal and resistant strains of this species, respectively. Data given under "Measured Activity" in each case represent median values from five replicate determinations from a single homogenate sample of 50 ml. containing the stated number of individuals, with the exception of a single determination only for the acid enzyme (check) determination for the adult drone honey bee.

Discussion

Check determinations of the acid enzyme compared with data of Rockstein and Levine (*op. cit.*) indicate a possible advantage of the cold-inactivation technique over the decapitation method previously employed. It should also be emphasized that *P. sericata* gives consistently very low *raw,* as well as *per weight,* data for both the acid and alkaline phosphatase when 50 individuals are homogenized. This is in contrast to the report by Rockstein and Levine (*op. cit.*) where the use of 25 individuals yielded raw data too low for accurate spectrophotometric determination by even this sensitive method.

In four species it is seen that the alkaline enzyme activity is lower than that of the acid phosphatase, both on a *raw data* and a *per weight* basis. An especially marked contrast is seen between the activities of these two enzymes in the case of the adult drone honey bee. This relationship is reversed in the case of *P. sericata,* while in the adult worker honey bee no appreciable difference between the two enzymes is apparent. Other studies (to be published) on these enzymes in relation to age in the adult worker honey bee indicate a marked increase in the acid enzyme activity within about ten days following emergence, while the alkaline enzyme shows a fall in activity after about the same period of time.

Differences in alkaline enzyme activity between male and female honey bees, male and female roaches, and between male and female flies, plus observed differences between resistant and normal house flies suggest that age, sex and variety are important factors to be considered in quantitative studies involving this enzyme system. The consistent difference in the alkaline enzyme activity of resistant and normal house flies suggests a possible relation of this enzyme

to the detoxification mechanism in DDT-resistant flies.

From a practical standpoint, scrutiny of the raw data indicates that all species employed, with the exception of *P. sericata,* would be useful for total homogenate studies of alkaline phosphatase. From a theoretical standpoint, it should be noted that the activity of the alkaline enzyme per gram of body weight, as compared to the acid enzyme, is consistently low in practically all species studied. The significance of such differences must await elaboration of the possible role of these enzymes, by further histochemical and biochemical studies; the sarcosome enzyme studies of Watanabe and Williams (1951) suggest another microbiochemical approach to this problem, by which parallel phosphatase studies might be made.

Acknowledgment

The authors acknowledge their appreciation to Dr. Horace S. Telford and Mr. Herman Menke for their kind cooperation in making available bees for this study. To Dr. A. W. Lindquist of the U.S.D.A., Corvallis, Oregon, are due thanks for his generous supply of house fly pupae of normal and resistant strains.

Summary

Alkaline phosphatase activity is reported for six different species of insects and compared with the acid enzyme. Raw data for the honey bee, house fly, American roach, large milkweed bug, and the yellow mealworm, indicate that these species are all suitable for quantitative studies involving this enzyme.

Literature Cited

Bradfield, J. R. G. 1946. Nature. 157: 876.
———— 1951. Qu. J. Microscop. Sci. 92: 87.
Day, M. F. 1949. Austral. J. Sci. Res. (B) 2: 31.
Denucé, J. M. 1952. Experientia 8: 64.
Moog, F. 1946. Biol. Rev. 21: 41.
Rockstein, M. and P. W. Herron. 1951. J. Cell. Comp. Physiol. 38: 451.
Rockstein, M. and L. Levine. 1951. Ann. Ent. Soc. Am. 44: 469.
Watanabe, M. I. and C. M. Williams. 1951. J. Gen. Physiol. 34: 675.
Yao, T. 1950a. Qu. J. Microscop. Sci. 91: 79.
———— 1950b. *Ibid.* 91: 89.